Claims:

1. A diagnostic or prognostic assay for a disease or condition in a subject, said disease or condition characterised by abnormal methylation of cytosine at a site or sites within the glutathione-S-transferase (GST) Pi gene and/or its regulatory flanking sequences, wherein said assay comprises the steps of;

(i) isolating DNA from said subject,

(ii) exposing said isolated DNA to reactants and conditions for the amplification of a target region of the GST-Pi gene and/or its regulatory flanking sequences which includes a site or sites at which abnormal cytosine methylation characteristic of the disease or condition occurs, the amplification being selective in that it only amplifies the target region if the said site or sites at which abnormal cytosine methylation occurs is/are methylated, and

(iii) determining the presence of amplified DNA,

wherein the amplifying step (ii) is used to amplify a target region within the region of the GST-Pi gene and/or its regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to +55.

2. An assay according to claim 1, wherein prior to the amplifying step, the isolated DNA is treated such that unmethylated cytosines are converted to uracil or another nucleotide capable of forming a base pair with adenine while methylated cytosines are unchanged or are converted to a nucleotide capable of forming a base pair with guanine.

3. As assay according to ~~any one of the preceding claims,~~ claim 1 wherein the amplifying step involves polymerase chain reaction (PCR) amplification.

4. An assay according to claim 3, wherein said PCR amplification utilises a reverse primer including guanine at at least one site whereby, upon the

reverse primer annealing to the treated DNA, said guanine will either form a base pair with a methylated cytosine (or another nucleotide to which the methylated cytosine has been converted through said treatment) if present, or will form a mismatch with uracil (or another nucleotide to which unmethylated cytosine has been converted through said treatment).

5. An assay according to claim 4, wherein said PCR amplification utilises a forward primer including cytosine at at least one site(s) corresponding to cytosine nucleotides that are abnormally methylated in the DNA of a subject with the disease or condition being assayed.

6. An assay according to claim 5, wherein the primers are of 12 to 30 nucleotides in length.

7. An assay according to claim 6, wherein the primers are selected so as to anneal to a sequence within the target region that includes two to four cytosine nucleotides that are abnormally methylated in the isolated DNA of a subject with the disease or condition being assayed.

8. An assay according to claim 2, wherein the treatment of the isolated DNA involves reacting the isolated DNA with bisulphite.

9. As assay according to claim 8, wherein the amplifying step involves polymerase chain reaction (PCR) amplification.

10. An assay according to claim 9, wherein said PCR amplification utilises a reverse primer including guanine at at least one site whereby, upon the reverse primer annealing to the treated DNA, said guanine will either form a base pair with a methylated cytosine if present, or will form a mismatch with uracil.

11. An assay according to claim 10, wherein said PCR amplification utilises a forward primer including cytosine at at least one site(s) corresponding to cytosine nucleotides that are abnormally methylated in the isolated DNA of a subject with the disease or condition being assayed.

12. An assay according to claim 11, wherein the primers are of 12 to 30 nucleotides in length.

13. An assay according to claim 12, wherein the primers are selected so as to anneal to a sequence within the target region that includes two to four cytosine nucleotides that are abnormally methylated in the DNA of a subject with the disease or condition being assayed.

14. An assay according to ~~any one of the preceding claims~~ claim 1, wherein said DNA is isolated from cells from tissue, blood (including serum and plasma), semen, urine, lymph or bone marrow.

15. An assay according to ~~any one of the preceding claims~~ claim 1, wherein the disease or condition to be assayed is selected from cancers.

16. An assay according to claim 15, wherein the disease or condition to be assayed is selected from prostate cancer, breast cancer, cervical cancer and liver cancer.

17. An assay according to claim 16, wherein the disease or condition to be assayed is prostate cancer.

18. An assay according to claim 17, wherein the amplifying step is used to amplify a target region within the region of the GST-Pi gene and its

regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to +53.

19. An assay according to claim 17, wherein the amplifying step is used to amplify a target region within the region of the GST-Pi gene and its regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to +10.

20. An assay according to claim 17, wherein the amplifying step is used to amplify a target region within the region of the GST-Pi gene and its regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to -14.

21. An assay according to claim 17, wherein the amplifying step is used to amplify a target region within the region of the GST-Pi gene and its regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to -8.

22. An assay according to ~~any one of the preceding claims~~ claim 1 wherein the target region excludes any or all of the CpG sites -36, -32, -23, -20, -19 and -14.

23. An assay according to ~~any one of~~ claims 5 ~~to 21,~~ wherein if either or both of the reverse or forward primers anneal to a sequence within the target region that includes any or all of CpG sites -36, -32, -23, -20, -19 and -14, then said PCR amplification further utilises equivalent reverse and/or forward primers including a redundant nucleotide(s) at the position(s) within their sequence(s) corresponding to cytosine or methylated cytosine of CpG sites -36, -32, -23, -20, -19 and -14.

24. An assay according to claim 17, wherein the amplifying step is used to amplify a target region within the region of the GST-Pi gene and its regulatory flanking sequences defined by (and inclusive of) CpG sites +9 to +53.

25. An assay according to claim 17, wherein the amplifying step is used to amplify a target region within the region of the GST-Pi gene and its regulatory flanking sequences defined by (and inclusive of) CpG sites +1 to +53.

26. An assay according to claim 17, wherein the amplifying step involves PCR amplification using primer pairs consisting of a forward and reverse primer selected from each of the following groups:

Forward Primers

CGCGAGGTTTTCGTTGGAGTTTCGTCGTC	(SEQ ID NO: 1)
CGTTATTAGTGAGTACGCGCGGTTC	(SEQ ID NO: 2)
YGGTTTTAGGGAATTTTTTTTCGC	(SEQ ID NO: 3)
YGGYGYGTTAGTTYGTTGYGTATATTTC	(SEQ ID NO: 4)
GGGAATTTTTTTTCGCGATGTTTYGGCGC	(SEQ ID NO: 5)
TTTTTAGGGGGTTYGGAGCGTTTC	(SEQ ID NO: 6)
GGTAGGTTGYGTTTATCGC	(SEQ ID NO: 7)

Reverse Primers

TCCCATCCCTCCCCGAAACGCTCCG	(SEQ ID NO: 8)
GAAACGCTCCGAACCCCCTAAAAACCGCTAACG	(SEQ ID NO: 9)
CRCCCTAAAATCCCCRAAATCRCCGCG	(SEQ ID NO: 10)
ACCCCRACRACCRCTACACCCCRAACGTCG	(SEQ ID NO: 11)
CTCTTCTAAAAAATCCCRCRAACTCCCGCCG	(SEQ ID NO: 12)
AAAACRCCCTAAAATCCCCGAAATCGCCG	(SEQ ID NO: 13)
AACTCCCRCCGACCCCAACCCCGACGACCG	(SEQ ID NO: 14)
AAAAATTCRAATCTCTCCGAATAAACG	(SEQ ID NO: 15)

AAAAACCRAAATAAAAACCACACGACG (SEQ ID NO: 16),

wherein Y is C, T or a mixture thereof, and R is A, G or a mixture thereof.

27. An assay according to claim 17, wherein the amplifying step involves PCR amplification using primer pairs consisting of a forward and reverse primer selected from each of the following groups:

Forward Primers

CGCGAGGTTTTCGTTGGAGTTTCGTCGTC (SEQ ID NO: 1)

CGTTATTAGTGAGTACGCGCGGTTC (SEQ ID NO: 2)

Reverse Primers

TCCCATCCCTCCCCGAAACGCTCCG (SEQ ID NO: 8)

GAAACGCTCCGAACCCCCTAAAAACCGCTAACG (SEQ ID NO: 9).

28. An assay according to claim 17, wherein the amplifying step involves PCR amplification using primer pairs consisting of a forward and reverse primer selected from each of the following groups:

Forward Primers

YGGTTTTAGGGAATTTTTTTTCGC (SEQ ID NO: 3)

YGGYGYGTTAGTTYGTTGYGTATATTTC (SEQ ID NO: 4)

GGGAATTTTTTTTTCGCGATGTTTYGGCGC (SEQ ID NO: 5)

Reverse Primers

CRCCCTAAAATCCCCRAAATCRCCGCG (SEQ ID NO: 10)

ACCCCRACRACCRCTACACCCCRAACGTCG (SEQ ID NO: 11)

CTCTTCTAAAAAATCCCRCRAACTCCCGCCG (SEQ ID NO: 12)

AAAACRCCCTAAAATCCCCGAAATCGCCG (SEQ ID NO: 13)

AACTCCCRCCGACCCCAACCCCGACGACCG (SEQ ID NO: 14),

wherein Y is C, T or a mixture thereof and R is A, G or a mixture thereof.

29. An assay according to claim 17, wherein the amplifying step involves PCR amplification using primer pairs consisting of a forward and reverse

primer selected from each of the following groups:

Forward Primers

TTTTTAGGGGGTTYGGAGCGTTTC (SEQ ID NO: 6)

GGTAGGTTGYGTTTATCGC (SEQ ID NO: 7)

Reverse Primers

AAAAATTCRAATCTCTCCGAATAAACG (SEQ ID NO: 15)

AAAAACCRAAATAAAAACCACACGACG (SEQ ID NO: 16),

wherein Y is C, T or a mixture thereof, and R is A, G or a mixture thereof.

30. An assay according to claim 16, wherein the disease or condition to be assayed is liver cancer.

31. An assay according to claim 30, wherein the amplifying step is used to amplify a target region within the region of the GST-Pi gene and its regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to -14.

32. An assay according to claim 31, wherein the target region excludes any or all of the CpG sites -36, -32, -23, -20, -19, and -14.

33. An assay according to claim 30, wherein if either or both of the reverse or forward primers anneal to a sequence within the target region that includes any or all of CpG sites -36, -32, -23, -20, -19 and -14, then said PCR amplification further utilises equivalent reverse and/or forward primers including a redundant nucleotide(s) at the position(s) within their sequence(s) corresponding to cytosine or methylated cytosine of CpG sites -36, -32, -23, -20, -19 and -14.

34. An assay according to claim 30, wherein the amplifying step is used to amplify a target region within the region of the GST-Pi gene and its

regulatory flanking sequences defined by (and inclusive of) CpG sites +9 to +53.

35. A diagnostic or prognostic assay for a disease or condition in a subject said disease or condition characterised by abnormal methylation of cytosine at a site or sites within the glutathione-S-transferase (GST) Pi gene and/or its regulatory flanking sequences, wherein said assay comprises the steps of;

(i) isolating DNA from said subject, and

(ii) determining the presence of abnormal methylation of cytosine at a site or sites within the region of the GST-Pi gene and/or its regulatory flanking sequences defined by (and inclusive of) CpG sites -43 to +55.

36. An assay according to claim 35, wherein the region of the GST-Pi gene and its regulatory flanking sequences within which the presence of methylated cytosine(s) at a site or sites is determined is selected from the regions defined by (and inclusive of) CpG sites -43 to +53, -43 to +10, -43 to -14, +9 to +53 and +1 to +53.

37. An assay according to claim 35 ~~or 36~~, wherein the said region of the GST-Pi gene and its regulatory flanking sequences excludes any or all of the CpG sites -36, -32, -23, -20, -19 and -14.

38. An assay according to claim 36, wherein the region of the GST-Pi gene and its regulatory flanking sequences within which the presence of methylated cytosine(s) at a site or sites is determined is the region defined by (and inclusive of) CpG sites +9 to +53.

39. An assay according to claim 36, wherein the region of the GST-Pi gene and its regulatory flanking sequences within which the presence of

methylated cytosine(s) at a site or sites is determined is the region defined by (and inclusive of) CpG sites +1 to +53.

40. An assay according to ~~any one of~~ claims 35 ~~to 39~~, wherein prior to the determination step, the isolated DNA is treated such that unmethylated cytosines are converted to uracil or another nucleotide capable of forming a base pair with adenine while methylated cytosines are unchanged or are converted to a nucleotide capable of forming a base pair with guanine.

41. An assay according to claim 40, wherein the treatment of the isolated DNA involves reacting the isolated DNA with bisulphite.

42. An assay according to ~~any one of~~ claims 35 ~~to 41~~, wherein the determination step involves selective hybridisation of oligonucleotide/polynucleotide/peptide-nucleic acid (PNA) probe(s).

43. An assay according to claim 42, wherein if the probe(s) hybridise to a sequence within the target region that includes any or all of CpG sites -36, -32, -23, -20, -19 and -14, then said selective hybridisations further utilises equivalent probe(s) including a redundant nucleotide(s) at the position(s) within their sequence(s) corresponding to cytosine or methylated cytosine of CpG sites -36, -32, -23, -20, -19 and -14.

44. An assay according to ~~any one of~~ claims 35 ~~to 43~~, wherein said DNA is isolated from cells from tissue, blood (including serum and plasma), semen, urine, lymph or bone marrow.

45. An assay according to ~~any one of~~ claims 35 ~~to 43~~, wherein the disease or condition to be assayed is selected from cancers.

46. An assay according to claim 45, wherein the disease or condition to be assayed is selected from prostate cancer, breast cancer, cervical cancer and liver cancer.

47. An assay according to claim 46, wherein the disease or condition to be assayed is prostate cancer.

48. An assay according to claim 46, wherein the disease or condition to be assayed is liver cancer.

49. A primer or probe comprising a nucleotide sequence selected from the group consisting of:

CGCGAGGTTTTCGTTGGAGTTTCGTCGTC	(SEQ ID NO: 1)
CGTTATTAGTGAGTACGCGCGGTTC	(SEQ ID NO: 2)
YGGTTTTAGGGAATTTTTTTTCGC	(SEQ ID NO: 3)
YGGYGYGTTAGTTYGTTGYGTATATTTC	(SEQ ID NO: 4)
GGGAATTTTTTTTCGCGATGTTTYGGCGC	(SEQ ID NO: 5)
TTTTTAGGGGGTTYGGAGCGTTTC	(SEQ ID NO: 6)
GGTAGGTTGYGTTTATCGC	(SEQ ID NO: 7)
AAAAATTCRAATCTCTCCGAATAAACG	(SEQ ID NO: 8)
AAAAACCRAAATAAAAACCACACGACG	(SEQ ID NO: 9)
TCCCATCCCTCCCCGAAACGCTCCG	(SEQ ID NO: 10)
GAAACGCTCCGAACCCCCTAAAAACCGCTAACG	(SEQ ID NO: 11)
CRCCCTAAAATCCCCRAAATCRCCGCG	(SEQ ID NO: 12)
ACCCCRACRACCRCTACACCCCRAACGTCG	(SEQ ID NO: 13)
CTCTTCTAAAAAATCCCRCRAACTCCCGCCG	(SEQ ID NO: 14)
AAAACRCCCTAAAATCCCCGAAATCGCCG	(SEQ ID NO: 15)
AACTCCCRCCGACCCCAACCCCGACGACCG,	(SEQ ID NO: 16),

wherein Y is a mixture of C and T, and R is a mixture of A and G.

50. A probe comprising a nucleotide sequence selected from the group consisting of:

AAACCTAAAAAATAAACAAACAA (SEQ ID NO: 17)

GGGCCTAGGGAGTAAACAGACAG (SEQ ID NO: 18)

CCTTTCCCTCTTTCCCARRTCCCCA (SEQ ID NO: 19)

TTTGGTATTTTTTTTTCGGGTTTTAG (SEQ ID NO: 20)

CTTGGCATCCTCCCCCGGGCTCCAG (SEQ ID NO: 21)

GGYAGGGAAGGGAGGYAGGGGYTGGG (SEQ ID NO: 22).